Importance of *Enterococcus* spp. for Forming a Biofilm

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Abstract: The aim of this study was to monitore the capability of *Enterococcus fecalis* and *Enterococcus fecium* to form biofilms. Enterococci isolates originated from individual milk, bulk milk samples and environmental swabs obtained at farm level, dairy plant level including semi and final dairy products. Biofilm formation potential was determined by growing the tested strains in glas tubes containing BHI medium. The capability of forming biofilms was detected in 28% of *Enterococcus* spp. strains. Higher number of biofilm forming strains of the *Enterococcus faecium* (33%) than *Enterococcus faecalis* (28%) has been registered. Isolates obtained at plant level were forming biofilms more often than isolates from plant level and in final products (cheese and curd cheese), no isolate has been seen to be able to form biofilm.

Keywords: biofilm; *Enterococcus fecalis; Enterococcus fecium*; dairy products; bacteria

INTRODUCTION

Permanently wet surfaces of the food industry facilities often support the adherence and colonisation of bacteria with subsequent formation of a biofilm. Bacteria in biofilm structures are more resistant not only to detergents but also against drying than planctonic ones (Bolton *et al*. 1988). Biofilm formation in food industry represents a serious issue (McLandsborough *et al*. 2006).

Nowadays, enterococci take up a significant position among the bacteria causing nosocomial infections. One of the main factors of enterococci virulence is the biofilm formation (Tendolkar *et al*. 2006). Enterococci are a part of common intestinal microflora in mammals and therefore they are often present in row material such as raw milk and meat.

The aim of this study was to assess the capability of forming biofilm in two enterococci species isolated from the samples at farm and dairy plant level.

MATERIAL AND METHODS

Enterococci isolates. Enterococci strains originating from the strain collection of Department of Hygiene and Milk Hygiene and Technology (University of Veterinary and Pharmaceutical Sciences, Brno) isolated from dairy chain in 2005–2007 and stored at –75°C were used in this study.

Genus and species identification of enterococci. Genus specific identification was carried out using the PCR method based on the detection of genus specific *tuf*-gene (Cupáková *et al*. 2005). *Enterococcus fecalis* and *Enterococcus fecium* identification was carried out using the PCR method based on the detection of genus specific sections of the *sod*A gene encoding the enzyme manganese-dependent superoxide dismutase (Jackson *et al*. 2004).

Detection of biofilm formation. For the assessment of the capability of biofilm formation in enterococci the phenotypic method was used. Suspension of tested strain was incubated in the glas tubes containing Brain Heart Infusion Broth (BHI

broth) aerobically at the temperature of 35°C for the period of 2 days. Than the supernatant was discarded, the glas tube has been stained by 0.1% safranin solution, washed with destilled water three times and dried. In the case of biofilm formation, a grainy red structure on the test tube bottom was found. All strains were tested in triplicates. The test tube finding was compared with the biofilm formation in reference strains (*Staphylococcus epidermidis* CCM 4418, CCM 7221).

RESULTS AND DISCUSSION

In total, 324 strains (303 strains of *Enterococcus faecalis* and 21 strains of *Enterococcus faecium*) were tested.

Enterococcus spp. biofilm formation capability was proven in 92 strains (28%), 232 strains (72%) was assessed to be biofilm negative.

From this study results, higher capability of biofilm formation can be seen in strains of the *Enterococcus faecium* species (Table 1). Monitoring of biofilm formation in *E. faecalis* and *E. faecium* was carried out by numerous authors who present variable results. Majority of the studies are focusing of the characteristics of clinical isolates from various sources. The conclusions of some of the studies indicate that *Enterococcus faecalis* forms biofilm more often than *Enterococcus faecium* (Prakash 2005; Di Rosa *et al*. 2006). The results of our study do not confirm this findings, however, the biofilm forming capability will most probably depend on the strains origin and the conditions of their cultivation.

Table 2 shows the distribution of biofilm positive enterococci according to the locality (farm and dairy plant). Isolates from farm level show a higher number of biofilm forming strains. The biofilm forming strains originated mainly from the bulk tank milk samples. Enterococci with both positive and negative biofilm formation were detected in swabs at the dairy plant level (production equipment, milk and cream samples prior to pasteurisation). In final products – cheese and curd cheese only strains incapable of biofilm formation were found. One of the reasons why enterococci isolated from the final products did not form biofilm

Table 1. Biofilm formation capability in the *Enterococcus faecalis* and *Enterococcus faecium* species

	Number of biofilm positive strains	Number of biofilm negative strains
Enterococcus faecalis	85 (28%)	218 (72%)
Enterococcus faecium	7 (33%)	14 (67%)

Table 2. *Enterococcus* spp. with a detected biofilm forming capability sorted by the sampling locations

Sample locality	Sample type	Number of biofilm positive strains			
		Enterococcus faecalis	*Enterococcus faecium*	Enterococci in total	
Farm 1	milk	9	0	9	19.5%
	swab	7	0	7	
Farm 2	milk	7	0	7	44.0%
	swab	4	0	4	
Farm 3	milk	11	0	11	34.7%
	swab	6	0	6	
Farm 4	milk	5	1	6	55.0%
	swab	3	2	5	
Dairy plant	swab	22	4	26	24.3%
	semi-products	6	0	6	
	final products	4	0	4	

could be due to the presence of sodium chloride in cheese (up to 4%) and a higher acidity of curd cheese (up to 70 SH). Several authors state that biofilm formation is impacted by the nutrient content in the cultivation medium, glucose, blood serum, iron, CO_2 and pH (Pillai *et al.* 2004). In *E. faecalis* as well as *E. faecium* strains isolated from three samples of butter and from one sample of whipped buttermilk, biofilm formation was confirmed.

CONCLUSION

A preventive measure against biofilm formation is the use of suitable production technologies. One of the repressive measure is efficient surface cleaning and sanitation of all the surfaces that come in touch with the raw materials.

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